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The Effect of Low-Intensity Exercise Training on Fat Metabolism of Obese Women

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Abstract

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Objective: Previous studies have shown that fat metabolism is different in upper body (UB) and lower body (LB) obese women. The present study investigated whether the effect of low-intensity exercise training on fat metabolism is different in UB and LB obese premenopausal women.

Research Methods and Procedures: Twenty-one healthy, premenopausal women with either LB obesity (waist-to-hip ratio of ≤ 0.79; $n = 8$) or UB obesity (waist-to-hip ratio of ≥ 0.85; $n = 13$) participated in the present study. The UB obese women were matched and randomly divided in an exercise training group (UB) and a nonexercising control group (UB-C). Subjects in the UB and LB groups participated in a low-intensity exercise training program (40% V_{O_2}max) three times per week for 12 weeks. Before and after the intervention, measurements of fat metabolism at rest and during exercise, body composition, and maximal aerobic capacity were performed.

Results: Exercise training did not change the respiratory exchange ratio at rest in the UB and LB groups. During exercise, relative fat oxidation increased in the UB group by 19% ($p < 0.05$), whereas no change in the LB and UB-C groups was found. Plasma free fatty acid oxidation did not change by exercise training, and nonplasma fatty acid oxidation tended to increase in the UB group compared with the UB-C group ($p = 0.08$).

Discussion: Low-intensity exercise training increased the contribution of fat oxidation to total energy expenditure during exercise but not at rest in UB obese women. Exercise training had no significant effect on fat metabolism in the LB obese women.

Key words: fat distribution, stable isotopes, acetate correction factor, upper body, lower body

Introduction

Human obesity has been classified by Vague (1) in a typically male or female type of fat distribution, called upper body (UB) or lower body (LB) obesity, respectively. However, both types of fat distribution may occur in obese women. UB obese women are known to have an increased risk of developing metabolic disorders such as insulin resistance, hypertension, and hyperlipidemia compared with LB obese women and nonobese women (2–4).

Body fat from the abdominal region is recognized as being much more lipolytic than adipose tissue from the gluteal-femoral regions (4,5). An in vivo study has shown that UB obese women, compared with LB obese or nonobese women, have an overall greater resting free fatty acid (FFA) mobilization (6–8), but FFA mobilization relative to fat mass was higher in nonobese women compared with UB obese women (8). LB obese women seem to have an increased ability to downregulate resting lipolysis to maintain normal FFA availability (7). However, respiratory exchange ratio (RER) at rest (9) and over a 24-hour period (10) and resting metabolic rate (9) are similar in UB and LB obese women. Epinephrine infusion (6) and an exercise session (11) showed a reduced lipolytic response in UB obese women compared with LB obese women and nonobese women. Therefore, fat oxidation and FFA availability during the total exercise session seem to be similar in UB and LB obese women (11,12). Furthermore, because UB obese women seem to have an impaired postabsorptive FFA utilization in skeletal muscle (13), FFA mobilization and oxidation do not seem to be coupled while FFA mobilization is elevated. Therefore, plasma FFA concentrations are elevated in UB obese women, and this elevation may be responsible for the fact that these women have an increased risk of developing metabolic complications (9). A study by

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Ranneries et al. (14) showed that obese subjects have an impaired ability to oxidize fat, even after weight loss has been achieved. An impaired ability to oxidize fat might predispose for weight gain (15,16). In Pima Indians, a population with a high prevalence of obesity, weight gain is associated with a low 24-hour ratio of fat to carbohydrate oxidation (17). Therefore, interventions to increase the capacity to oxidize FFA in obese individuals are important in weight management and in reducing the risk for metabolic complications associated with obesity.

Endurance exercise training is known to increase fat oxidation during submaximal exercise at a fixed workload in lean subjects (18–22). A recent study at our laboratory showed that 12 weeks of low-intensity exercise training can induce an increase in fat oxidation during exercise in obese men independent of weight changes (23). Only a few studies have reported effects of exercise training on fat oxidation in lean women (19,24) showing an increased fat oxidation after training. However, to our knowledge, no study on the effect of exercise training on fat oxidation in obese women has been performed so far. Because of the differences in fat metabolism between UB and LB obese women described above, it seems to be important to distinguish the effects of exercise training in UB and LB obese women. The present study was undertaken to investigate the effects of low-intensity exercise training on fat metabolism in obese women and to compare the effects in UB and LB obese women. At recruitment for this exercise training study, subjects were told that no voluntary energy restriction was allowed during the study because changes in body weight affect fat metabolism and therefore interfere with the effect of exercise training on fat metabolism.

Research Methods and Procedures

Subjects

Twenty-one obese female subjects (body mass index [BMI] of $\geq$29 kg/m^2) with either LB obesity ($n = 8$; waist-to-hip ratio [WHR] of $\leq$0.79) or UB obesity ($n = 13$; WHR of $\geq$0.85) participated in this study. Subjects were recruited by advertisements in local newspapers. The subjects were premenopausal and reported to have a normal menstrual cycle. Subjects with UB obesity were matched in pairs based on age, BMI, and maximal oxygen uptake per kilogram of fat-free mass and were randomly divided into an exercise training group (UB) ($n = 7$) or a nontraining control group (UB-C) ($n = 6$). Because recruitment of LB women was difficult, the LB group was too small to divide into a training and nontraining group. Therefore, all subjects in the LB group participated in the exercise training program ($n = 8$). Physical characteristics are given in Table 1. All subjects were in good health as assessed by medical history and physical examination and were weight-stable for at least the previous 2 months ($<$3 kg weight change). None of the subjects received medication known to affect the variables measured. The subjects did not spend $>$2 hours per week in sports activities and did not have a physically demanding job. Subjects were requested to maintain their dietary habits during the study. The Ethics Committee of Maastricht University approved the study protocol. Written informed consent was obtained from all subjects.

Experimental Design

Two of the three groups (LB and UB) participated in an exercise training intervention of 12 weeks. The third group served as a nontraining control group (UB-C). Measurements of body composition, maximal aerobic capacity, and fat metabolism were made before the start of the exercise training program and repeated within 2 weeks after 12 weeks of exercise training. Measurements of fat metabolism were performed in the midfollicular phase of the menstrual cycle. The exercise training program was continued until all measurements were performed.

Exercise Training

The exercise training program consisted of cycling on an ergometer (Bodyguard Cardiocycle, Sandnes, Norway; or Excalibur; Lode, Groningen, The Netherlands) at low intensity (40% V_{O_2}max). Eight LB obese women and seven UB obese women participated in the exercise training program. Subjects trained for at least 12 weeks, three times per week. Because measurements of fat metabolism were performed in the midfollicular phase, the exact duration of the training program was dependent on the length of the menstrual cycle. Energy expenditure of each subject in each training session was set at 5 kcal/kg of fat-free mass ($\sim$250 kcal). The average training duration was 57 $\pm$ 6 minutes. Heart rate was monitored continuously during the training sessions (Polar Electro, Oy, Finland). After 4 and 8 weeks of exercise training, training intensity was checked by a maximal aerobic exercise test and adjusted if necessary. All training sessions took place at the university under the supervision of a professional instructor.

Measurements

Body Composition. Subjects were weighed on a digital balance accurate to 0.1 kg (model D-7470; Sauter, Ebingen, Germany). Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (model 220; Seca, Hamburg, Germany). Body density was measured by hydrostatic weighing, with a correction for residual lung volume measured by helium dilution with a spirometer (Volugraph 2000, Mijnhardt, The Netherlands) at the time of underwater weighing. Body composition was calculated according to the formula of Siri (25).

Fat distribution was estimated by measuring WHR with a flexible, nonelastic tape measure with the subject in standing position. All measurements were recorded to the nearest

Table 1. Subject characteristics before and after the intervention period in the LB ($n = 8$), UB ($n = 7$), and UB-C ($n = 6$) groups

	LB		UB		UB-C	
	Before	**After**	**Before**	**After**	**Before**	**After**
Age (years)	32.8 ± 9.6		37.7 ± 6.4		42.5 ± 6.4	
Body weight (kg)	91.2 ± 9.7	91.2 ± 9.3	86.5 ± 10.2	87.1 ± 10.1	94.7 ± 14.0	94.5 ± 14.5
BMI (kg/m^2)	32.8 ± 3.9	32.9 ± 3.6	32.1 ± 2.9	32.4 ± 3.0	33.3 ± 3.8	33.1 ± 3.9
Body fat (%)	45.0 ± 4.4	45.9 ± 4.3	42.6 ± 3.1	42.8 ± 2.4	44.4 ± 3.0	44.9 ± 3.2
WHR	0.75 ± 0.03	0.75 ± 0.04	0.89 ± 0.04†	0.89 ± 0.05	0.90 ± 0.03	0.88 ± 0.03
Fat-free mass (kg)	50.0 ± 2.4	49.5 ± 2.7	49.4 ± 3.7	49.6 ± 3.8	52.5 ± 6.1	51.8 ± 5.8
V_{O_2}max (ml/min)	2251 ± 253	2536 ± 162*‡	2126 ± 168	2188 ± 291	1913 ± 460	1966 ± 359
V_{O_2}max/Fat-free mass (ml/kg per minute)	45.1 ± 5.1	51.3 ± 3.8*‡	43.2 ± 4.0	44.0 ± 3.1	36.7 ± 8.6	37.4 ± 6.3

Values are expressed as means ± SD.
* Significantly different from before ($p < 0.05$).
† Significantly different from LB before ($p < 0.01$).
‡ Change significantly different from UB ($p < 0.05$).

0.1 cm and the mean of two measurements was calculated. Measurements were performed after a normal exhalation with the subject in standing position, the abdomen relaxed, the arms at the sides, and the feet together. Waist circumference was measured at the smallest circumference between the ribs and iliac crest. Hip circumference was measured at the level of maximal extension of the buttocks (26).

Maximal Aerobic Capacity. Maximal oxygen uptake (V_{O_2}max) for each subject was determined by an incremental exercise test on an electromagnetically braked cycle ergometer (Excalibur). After a warm-up period of 4 minutes at 50 W, workload was increased every 3 minutes by 30 W until exhaustion. During the experiment, ventilatory and gas exchange responses were measured continuously using indirect calorimetry (Oxycon β, Mijnhardt, The Netherlands). Heart rate was recorded continuously by an electrocardiogram. The highest oxygen uptake achieved over 30 seconds was taken as V_{O_2}max.

Measurements of Fat Oxidation and Rate of Appearance of FFA During Rest and Exercise. Fat metabolism was studied by means of indirect calorimetry and stable isotope tracer methodology. To study fat metabolism, all subjects participated in two tracer tests before and after the training intervention in which palmitate and acetate were infused, respectively. The acetate infusion test was performed to obtain a correction factor for the loss of ^{13}C label in the tricarboxylic acid (TCA) cycle. The tracer tests were separated by 1 week to prevent carryover of the label. The sequence of the tracer tests was random. Subjects filled in a food and exercise questionnaire 3 days before the first tracer test. They were instructed to follow the same food and exercise habits 3 days before the second tracer test and the tracer tests after the training intervention to exclude bias by these factors.

[*U-^{13}C]-Palmitate Infusion.* This experiment was performed at least 36 hours and at the most 64 hours after the last exercise session with subjects in the midfollicular phase of the menstrual cycle. Subjects were asked to refrain from consumption of naturally ^{13}C-enriched food products such as cane sugar and corn for 1 week before the experiment. After an overnight fast, subjects came to the laboratory by car or bus. Subjects remained in semisupine position throughout the first 2.5 hours of the experiment. Catheters were inserted in an arm vein for infusion of the palmitate tracer and retrogradely into a contralateral dorsal hand vein for blood sampling. The cannulated hand was placed in a hot box, in which warm air of 60 °C circulated, to obtain arterialized venous blood. A baseline arterialized blood sample was taken after 30 minutes. Baseline expired breath was sampled in a 15-mL vacutainer tube (Becton Dickinson, Meyland, France) to determine the background enrichment. Immediately after baseline samples were taken, subjects were given an intravenous dose of 1.0 μM/kg NaH $^{13}CO_3$ to prime the bicarbonate pool. Next, a constant infusion of 0.0062 μM/kg per minute of [U-^{13}C]-palmitate was started ($t = 0$), using an IVAC pump (IVAC Medical, Amersfoort, The Netherlands). This infusion was continued for 120 minutes, with the subject in semisupine position. Subsequently, subjects started to exercise in a sitting position for 1 hour at 50% of pretraining V_{O_2}max on a cycle ergometer (Excalibur). The infusion rate during exercise was doubled to minimize changes in isotopic enrichment. At rest V_{CO_2}

and V_{O_2} were measured using an open-circuit ventilated hood system and a mouthpiece was used during exercise (Oxycon β). At rest and during exercise, V_{CO_2} and V_{O_2} were measured during the 5-minute period immediately before taking a breath sample for measurement of $^{13}CO_2$ enrichment. The accuracy of the system for measuring V_{CO_2} and V_{O_2} was tested regularly to be within 5%. Breath samples were taken at $t = 100$, 110, and 120 minutes at rest and at $t = 40$, 50, and 60 minutes during exercise. The exact infusion rate of [U-^{13}C]-palmitate was determined for each experiment by measuring the concentrations of the infusates (see Sample Analysis). Blood samples were taken at $t = 100$, 110, and 120 minutes of rest and at $t = 40$, 50, and 60 minutes during exercise. Blood samples were put into chilled 10-mL tubes containing EDTA or heparin plus 300 μL of glutathione (45 μg/L saline) and immediately centrifuged at 800 $\times$ *g* for 10 minutes at 4 °C. Plasma was stored at −80 °C until analysis. The EDTA-containing blood was used for analyses of plasma glucose, FFA, insulin, triglyceride (TG), and palmitate concentrations as well as the plasma enrichment of palmitate. The heparin- and glutathione-containing blood was used for analysis of plasma catecholamines. During rest ($t = 0$, 90, and 120 minutes) and exercise ($t = 30$ and 60 minutes), blood was sampled for the measurement of oxygen saturation (Hemoxymeter OSM2, Copenhagen, Denmark) to check the arterialization. Before infusion, the palmitate tracer (60 mg of potassium salt of [U-^{13}C]-palmitate, enrichment 98.9%; Cambridge Isotope Laboratories, Andover, MA) was bound to albumin by dissolving it in heated (60 °C) sterile water and passing it through a 0.2-μm filter into a 5% warm (60 °C) human serum albumin solution to make a 0.670-mM solution. *[1,2-^{13}C]-Acetate Infusion.* Palmitate oxidation rates were corrected for loss of tracer in products of the TCA cycle using the acetate correction factor described previously by Sidossis et al. (27) and Schrauwen et al. (28). The protocol for the acetate infusion experiment was the same as for the palmitate infusion experiment, except that no blood was sampled. The acetate tracer (sodium salt of [1,2-^{13}C]-acetate, enrichment 99%; Cambridge Isotope Laboratories) was dissolved in 0.9% saline. The acetate infusion rate was 0.0496 $\mu M \cdot mol \cdot kg^{-1} \cdot min^{-1}$ at rest and was doubled during exercise. Before starting the acetate infusion, an intravenous dose of 1.0 μM/kg NaH $^{13}CO_3$ was given to prime the bicarbonate pool.

Sample Analysis

Plasma total FFA, glucose, glycerol, and TG concentrations were measured on a Cobas Fara centrifugal spectrophotometer (Hoffmann-La Roche, Basel, Switzerland). For analysis of plasma total FFA concentrations, a non-esterified fatty acids colorimetric determination kit (Wako Chemicals, Neuss, Germany) was used. Plasma glucose concentrations were measured with a glucose hexokinase kit (Hoffman-La Roche). Plasma glycerol and TG concentrations were analyzed using a glycerol kit (Boehringer Mannheim, Mannheim, Germany). Plasmas with known concentrations were included in each run for quality control. Plasma insulin concentrations were measured with a double-antibody radioimmunoassay (Insulin RIA 100; Pharmacia, Uppsala, Sweden). Plasma catecholamine concentrations were analyzed by high-performance liquid chromatography with electrochemical detection (29).

The chemical and isotopic purity (99%) of the palmitate and acetate tracers were checked by ^{1}H and ^{13}C nuclear magnetic resonance and gas chromatography (GC)/mass spectrometry. Breath samples were analyzed for $^{13}C/^{12}C$ ratio using a GC-isotope ratio mass spectrometer (Finnigan MAT 252, Bremen, Germany). For determination of the plasma palmitate concentration, FFA was extracted from plasma, isolated by thin-layer chromatography, and derivatized to methyl esters. Palmitate concentration was determined on an analytical gas chromatograph with flame ionization detection using heptadecanoic acid as an internal standard. The isotope tracer/tracee ratio (TTR) of palmitate was determined using a GC combustion isotope ratio mass spectrometer, with correction for the extra methyl group in the derivative.

The concentration of the acetate infusate was determined on a Cobas Fara centrifugal spectrophotometer with an enzymatic method kit (no. 148261; Boehringer Mannheim). The concentration of the palmitate infusate was determined as described above for plasma samples.

Calculations

Total fat oxidation was calculated using the following equation (30): total fat oxidation (g/min) = $1.67 \times V_{O_2} - 1.67 \times V_{CO_2}$, with V_{CO_2} and V_{O_2} in liters per minute. Total fatty acid (FA) oxidation was determined by converting the rate of total fat oxidation to its molar equivalent, with the assumption that the average molecular weight of TG is 860 g/mol, and multiplying the molar rate of TG oxidation by 3 because each molecule contains 3 mol of fatty acids.

^{13}C enrichment of breath CO_2 and plasma palmitate is expressed as TTR. TTR was defined as follows: $(^{13}C/^{12}C)_{sa} - (^{13}C/^{12}C)_{bk}$, in which sa equals sample and bk equals background.

Fractional recovery of infused acetate ^{13}C label in breath CO_2 was calculated using the following formula: Acetate recovery = (TTR $CO_2 \times V_{CO_2}$)/F, where F is infusion rate mM/min).

The rate of [U-^{13}C]-palmitate oxidation was calculated as follows: Plasma palmitate oxidation (μM/min) = (TTR $CO_2 \times V_{CO_2}$)/(TTRplasma $\times$ acetate recovery) $\times$ 1000. The average V_{CO_2} over the last three time points at rest and during exercise was used.

The total plasma FA oxidation was then calculated by dividing palmitate oxidation by the fractional contribution of palmitate to the total FFA concentration. The average

fraction of palmitate per FFA was used in this equation, and total plasma FA oxidation per time point was calculated over the rest and exercise period.

Nonplasma-derived FA oxidation (μM/min), which refers to intramuscular TGs (IMTGs) and plasma triacylglycerol, was calculated at rest and during exercise as the average total FA oxidation minus the average plasma FFA oxidation.

Rate of appearance of palmitate in plasma, which under steady-state conditions is equal to the rate of disappearance minus tracer infusion rate, was calculated as follows: Rate of appearance (μM/min) = ([TTRinfusate/TTRplasma] − 1) × F. The percentage of plasma FFA cleared from the circulation that was oxidized was calculated using the following formula: % Rate of appearance of oxidized FFA = plasma FFA oxidation/Rate of appearance of FFA.

Statistics

Data are expressed as means ± SD. Differences between the LB and UB groups and between the UB and UB-C groups were tested by the Mann–Whitney test. Changes within groups were analyzed by the Wilcoxon signed rank test. A p value of <0.05 was regarded as statistically significant.

Results

Subject Characteristics

Before the intervention, subject characteristics were not significantly different between groups, except for the WHR, which was, as intended, significantly higher in the UB group compared with the LB group ($p < 0.01$) (Table 1). Exercise training did not significantly affect body weight or body composition in any group. In the LB group, exercise training induced a significant increase in V_{O_2}max and V_{O_2}max per kilogram of fat-free mass ($p < 0.05$) and the effect in the LB group was significantly different from that in the UB group ($p < 0.05$). The absolute workloads during the exercise test (50% of pretraining V_{O_2}max) in the LB, UB, and UB-C group were 65 ± 18, 55 ± 11, and 45 ± 30 W, respectively. Workloads were not significantly different between the LB and UB groups and between the UB and UB-C groups. Attendance at the exercise training sessions was 81 ± 19% for the LB group and 88 ± 17% for the UB group.

Rest

Data from indirect calorimetry showed that relative fat oxidation during the last 20 minutes of the resting period, expressed as RER, did not change due to the intervention in any group (Table 2). Total FA oxidation, energy expenditure (Table 2), and percentage of fat oxidation of total energy expenditure (Figure 1A) did not change in any of the groups either.

Plasma palmitate enrichment was at a plateau (change, 1.6%) during the last 20 minutes of the resting period. Therefore, tracer calculations of plasma palmitate oxidation were made using the values of plasma palmitate enrichment measured over the 100- to 120-minute period. These tracer calculations were corrected by acetate recovery factors that were measured over the same time points. The fractional recovery of acetate before the intervention increased gradually at rest from 22.2 ± 1.9% at 100 minutes to 25.4 ± 2.2% at 120 minutes after the start of the tracer infusion. Exercise training did not significantly influence the acetate recovery factor. Exercise training did not affect resting plasma FFA oxidation (Figure 1B), nonplasma FA oxidation (plasma triacylglycerol and IMTGs; calculated as total FA oxidation minus plasma FFA oxidation) (Figure 1C), rate of appearance of FFA (Figure 2A), and FFA oxidation as a percentage of the rate of appearance of FFA (Figure 2A, numbers between brackets). Nonplasma FA oxidation at rest was significantly lower than zero before and after exercise training ($p < 0.05$).

Average plasma concentrations (mean of last 20 minutes at rest) of FFA (Figure 3A) and glycerol (Figure 3B) were not significantly different after the intervention compared with before the intervention. Plasma TG concentrations before the intervention were significantly lower in the LB group compared with the UB group ($p < 0.05$) (Figure 3C). Exercise training did not affect plasma TG concentrations. Plasma epinephrine and norepinephrine concentrations were unchanged after the intervention (Table 3). Plasma insulin concentration was significantly increased in the LB group after the intervention ($p < 0.05$) but did not change in the UB and UB-C groups (Table 3). However, the change in the LB group was not significantly different from that observed for the UB group. Plasma glucose concentrations did not change over the intervention period (data not shown).

Exercise

During the last 20 minutes of exercise, RER was significantly decreased in the UB group after exercise training ($p < 0.05$), whereas RER did not change in the LB and UB-C groups. The change in RER in the UB group was significantly different from the change in the LB and UB-C groups ($p < 0.05$) (Table 2 and expressed as percentage of fat oxidation of total energy expenditure in Figure 1D). Because energy expenditure significantly decreased in the UB group ($p < 0.05$), total FA oxidation did not change significantly in the UB group (Table 2).

Plasma palmitate enrichment was at a plateau (change, 1.1%) during the last 20 minutes of the exercise period. The acetate correction factor increased gradually during exercise from 60.0 ± 7.5% after 40 minutes to 63.5 ± 8.0% after 60 minutes of exercise at 50% V_{O_2}max before the intervention. The intervention did not change the acetate recovery factor. The plasma FFA oxidation before the intervention was not

Table 2. Energy expenditure and substrate oxidation results from indirect calorimetry at rest and during exercise before and after the intervention period in the LB, UB, and UB-C groups

	LB		UB		UB-C	
	Before	**After**	**Before**	**After**	**Before**	**After**
Rest						
RER	0.82 ± 0.02	0.83 ± 0.02	0.82 ± 0.03	0.83 ± 0.03	0.80 ± 0.04	0.82 ± 0.04
EE (kcal/min)	1.19 ± 0.09	1.19 ± 0.09	1.10 ± 0.08	1.12 ± 0.13	1.14 ± 0.13	1.15 ± 0.15
Total FA oxidation (μmol/min)	269 ± 49	249 ± 27	248 ± 51	239 ± 61	280 ± 42	252 ± 63
Exercise						
RER	0.82 ± 0.03	0.84 ± 0.03	0.85 ± 0.02	0.81 ± 0.02*†	0.86 ± 0.05	0.86 ± 0.07
EE (kcal/min)	6.00 ± 1.16	5.73 ± 0.99	5.20 ± 0.61	4.99 ± 0.58	4.91 ± 1.46	5.16 ± 1.70
Total FA oxidation (μmol/min)	1286 ± 365	1127 ± 224	970 ± 171	1151 ± 134	815 ± 292	813 ± 421

EE, energy expenditure. Values are expressed as means ± SD.
* Significantly different from before ($p < 0.05$).
† Change significantly different from LB and UB-C ($p < 0.05$).

different between groups and was not affected by exercise training (Figure 1E). The nonplasma FA oxidation during exercise was not significantly different from zero (Figure 1F). Exercise training did not significantly influence nonplasma FA oxidation, although the change in nonplasma FA oxidation tended to be different in the UB group compared with the UB-C group ($p = 0.08$). Neither the rate of appearance of FFA (Figure 2B) nor the percentage of FFA oxidized from the rate of appearance of FFA (Figure 2B, numbers between brackets) changed due to the intervention.

The plasma FFA concentrations after exercise training were significantly lower than before in the LB group ($p < 0.05$) (Figure 3D). However, concentrations before the intervention and the changes due to the intervention were not significantly different between groups. The plasma glycerol concentrations were not significantly different between groups before the intervention (Figure 3E). Plasma glycerol concentrations tended to decrease after the intervention in the LB group ($p = 0.07$). Changes were not different between groups. Plasma TG concentrations were significantly higher in the UB group compared with the LB group ($p < 0.05$) (Figure 3F). Exercise training did not affect plasma TG concentrations significantly. Plasma glucose concentrations did not change (data not shown). Training plasma epinephrine, norepinephrine, and insulin concentrations were not different from what was observed before exercise (Table 3).

Discussion

The major new finding of the present study is that low-intensity exercise training increases the contribution of fat oxidation to total energy expenditure during exercise in premenopausal UB obese women, but not in LB obese women.

Low-intensity exercise training increased the relative fat oxidation during exercise in the UB group by 19% ($p < 0.05$), whereas it remained unchanged in the LB and UB-C groups. In addition, the change in relative fat oxidation during exercise in the UB group was significantly different from the change in the LB and UB-C groups ($p < 0.05$). This means that fat oxidation at a certain energy expenditure was increased in the UB group but not in the LB and UB-C groups. Because energy expenditure was lower during exercise after training in the UB group ($p < 0.05$), only the relative amount of fat oxidation increased, not the absolute amount. The lower energy expenditure in the UB group during exercise after training might be explained by an increased efficiency during cycling due to the cycling training, which is also apparent in the LB training group (not significant) but not in the nontraining control group. The variability in energy expenditure during exercise between groups is explained by differences in absolute workload. In a previous study in which obese men executed the same protocol as the women in the present study, we also found an increased relative fat oxidation during exercise after training (23). Because fat distribution in obese men was comparable with UB women, this suggests that fat distribution, and associated metabolic features, plays an important role in the effect of low-intensity exercise training on fat oxidation, which is also suggested by Krotkiewski and Björntorp (31).

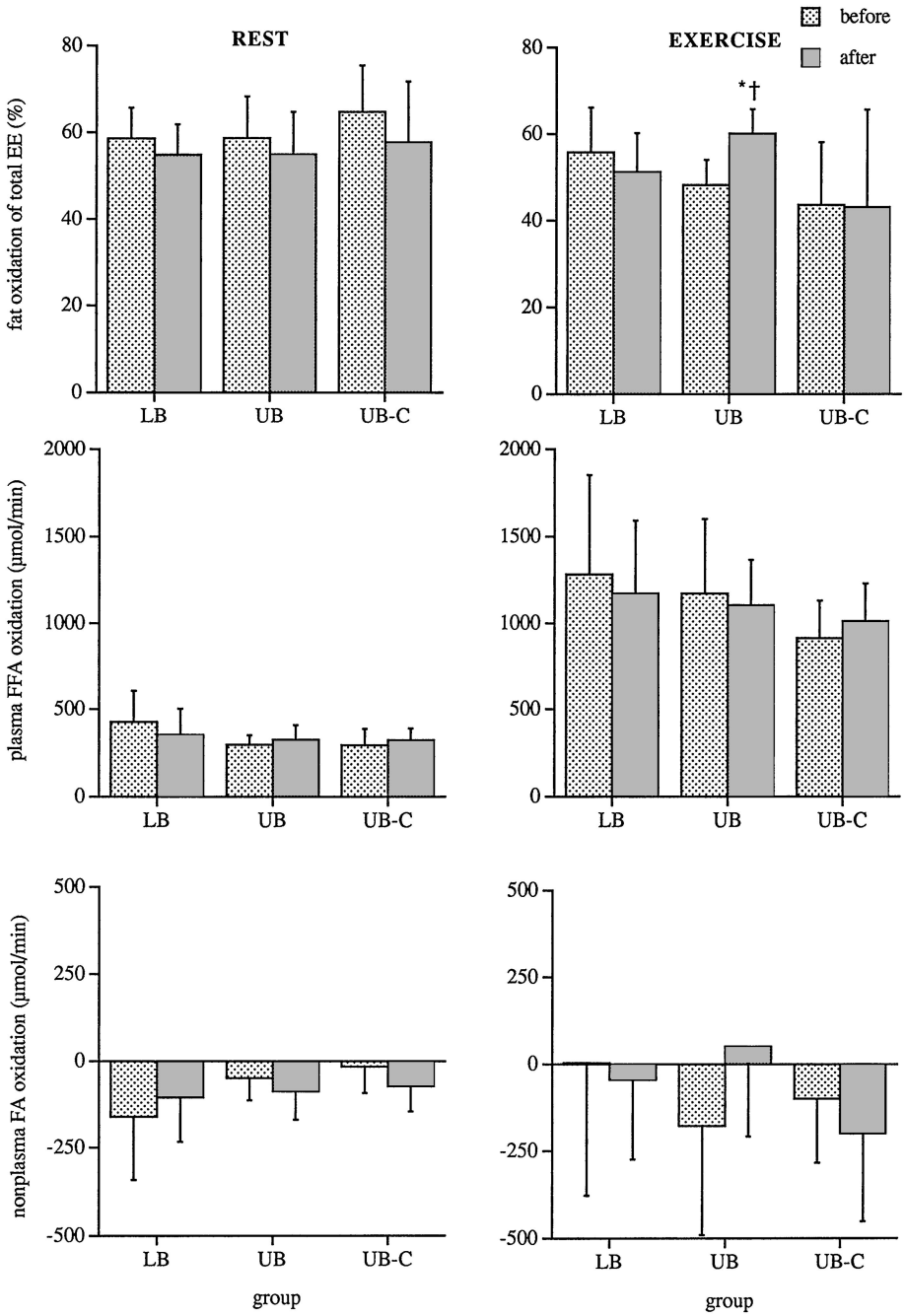

Figure 1. Fat oxidation as a percentage of total energy expenditure (EE), plasma FFA oxidation, and nonplasma FA oxidation (IMTG and VLDL-TG) over the last 20 minutes during rest (A, B, and C, respectively) and exercise (D, E, and F, respectively) in the LB, UB, UB-C groups before and after the intervention. *Significantly different from before ($p < 0.05$); †Significantly different from the LB and UB-C groups.

The increase in relative fat oxidation in the UB group after training is more likely caused by an increase in nonplasma FA oxidation (increased nonsignificantly) than by changes in plasma FFA oxidation (decreased slightly). However, determination of a significant increase in nonplasma FA oxidation is difficult due to the low values, the high variability, and the indirect measurement method. Lipolysis from adipose tissue did not seem to change with exercise training because the rate of appearance of FFA, plasma FFA, and glycerol concentrations did not differ from what was observed before the intervention. This can be supported by unchanged plasma catecholamine concentra-

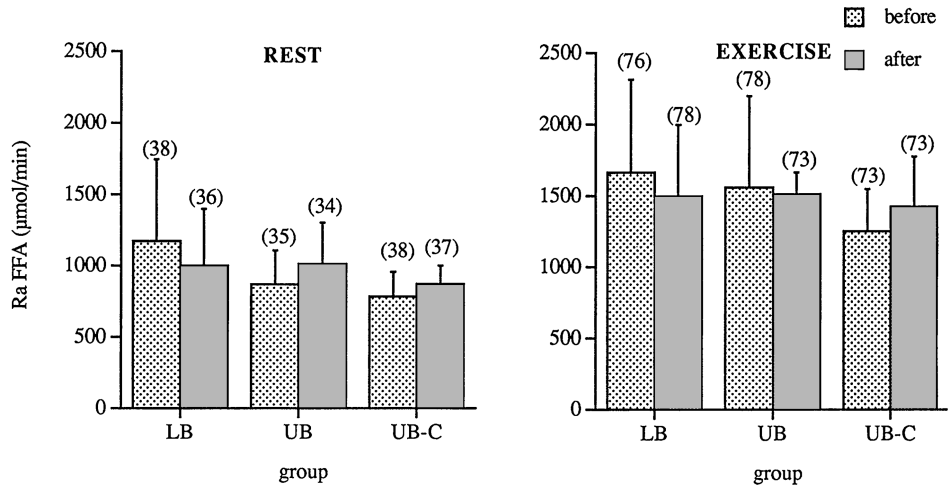

Figure 2. Rate of appearance (Ra) of FFA (μM/min) in the LB, UB, and UB-C groups at rest (A) and during exercise (B) before and after the intervention. Numbers between brackets indicate the % Rate of appearance of oxidized FFA.

tions, which play an important role in the regulation of lipolysis. Although nonplasma FA oxidation did not change significantly in the UB group ($p = 0.25$), the change tended to be significant compared with the change in the UB-C group ($p = 0.08$). An increase in nonplasma FA oxidation in the UB group would be in line with similar results found in obese men after the same exercise training protocol (our unpublished results). An increase in nonplasma FA oxidation after exercise training could implicate either increased IMTG oxidation, increased very low-density lipoprotein (VLDL)-TG oxidation, or both. The data in the present study cannot distinguish between the two. No consistent data on training effects on VLDL-TG or IMTG oxidation are available in the literature. Because the increased fat oxidation in UB obese women seems to be coming from nonplasma FA pools (IMTG and VLDL-TG) rather than from plasma FFA (lipolysis from adipose tissue), the question can be raised as to whether training will help to reduce adipose mass in the UB obese women. However, the depleted nonplasma FA pools after exercise might be restored by FFA from adipose tissue. Whether this increase in fat utilization during exercise in the UB group contributes to positive/favorable effects of exercise training on body mass remains to be studied. Exercise training in obese women has been reported to result in a comparatively greater loss of fat from the abdominal region compared with the gluteal region as measured by computed tomography (32).

Similarly, there was a significant decrease in WHR after 6 months of exercise training in obese young women who lost weight, as well as in those who did not lose weight as a result of exercise training (33). This suggests a greater fat loss from abdominal fat depots compared with gluteal fat due to exercise training. Although subjects in the present study did not lose weight or fat mass and WHR did not change due to exercise training, the increase in fat oxidation in the UB group might suggest that more fat was mobilized from the abdominal fat depot compared with the gluteal fat depot due to exercise training. The different effect of exercise training in UB compared with LB obese women can probably not be explained by a different effect of exercise training on muscle morphology, because no changes in muscle morphology were reported after exercise training in UB and LB obese women (31).

Preintervention data from our study show no difference in fat metabolism between UB and LB obese women at rest and during exercise. In agreement with our data, others also showed no difference in total fat oxidation between UB and LB obese women at rest (9), over a 24-hour period (10), and over a total exercise session (11,12). However, the increase in FFA availability during exercise was reported to be higher in LB obese women (11). Furthermore, we found that the rate of appearance of FFA and plasma concentrations of FFA and glycerol were similar in UB and LB obese women. This does not agree with the findings of others, who report an increased basal lipolysis in UB obese women compared with LB obese women (6,7). However, the significantly higher level of circulating TG in UB obese women compared with LB obese women, also reported by Jensen (8), might indicate a low adipose tissue lipoprotein lipase activity in UB obese women, which is in agreement with data reported by others (34,35).

In the present study, the fasting plasma insulin concentration increased in the LB obese group after exercise training ($p < 0.05$). There was no change in the UB and UB-C groups. We have no explanation for the increased plasma insulin concentration in the LB group. Others showed a decrease in plasma insulin after exercise training without weight loss in obese and patients with coronary artery

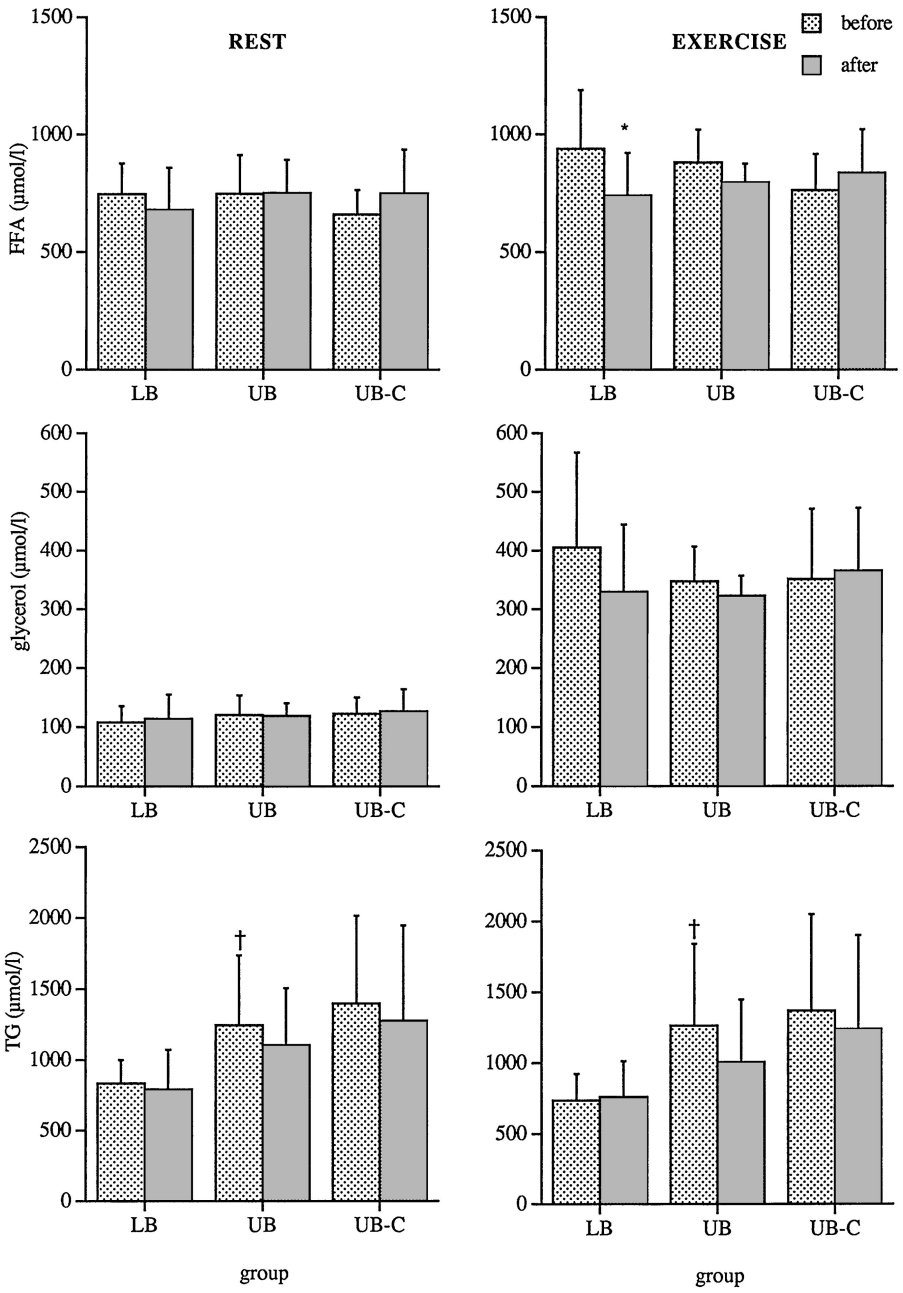

Figure 3. Plasma FFA, glycerol, and TG concentrations over the last 20 minutes during rest (A, B, and C, respectively) and exercise (D, E, and F, respectively) in the LB, UB, and UB-C groups before and after the intervention. *Significantly different from before ($p < 0.05$); †Significantly different from LB before ($p < 0.05$).

disease (36–38). It remains to be clarified whether this increase in plasma insulin concentration is accidental or typical for LB obese women.

Low-intensity exercise training was shown to have an effect on the relative fat oxidation of UB obese women. However, no effect of exercise training could be found on the V_{O_2}max of the UB obese women, but V_{O_2}max did increase in the LB obese women. Nevertheless, the exercise training program, performance during the maximal aerobic capacity test, and attendance at the training sessions were similar in both groups. We do not have an explanation for the lack of improvement of V_{O_2}max in the UB obese women. Apparently, the changes in fat metabolism found in the present study are independent of changes in V_{O_2}max.

Methodological Considerations

In the present study, only a UB obese control group participated; due to a lack of LB participants, there was no

Table 3. Average plasma concentrations of epinephrine (ng/liter), norepinephrine (ng/liter), and insulin (μU/ml) in the LB, UB, and UB-C groups before and after the intervention

	Epinephrine		Norepinephrine		Insulin	
Group	**Rest**	**Exercise**	**Rest**	**Exercise**	**Rest**	**Exercise**
LB before	33 ± 9	70 ± 11	271 ± 91	1008 ± 318	8.1 ± 1.8	7.1 ± 1.4
LB after	32 ± 9	71 ± 34	299 ± 85	910 ± 300	9.6 ± 2.0*	8.3 ± 2.6
UB before	36 ± 10	100 ± 81	339 ± 119	949 ± 293	10.2 ± 3.2	8.0 ± 2.5
UB after	41 ± 18	74 ± 41	330 ± 87	874 ± 243	9.5 ± 3.1	7.5 ± 1.8
UB-C before	34 ± 14	60 ± 37	335 ± 69	950 ± 542	12.2 ± 6.8	9.9 ± 3.7
UB-C after	34 ± 18	73 ± 49	324 ± 80	999 ± 547	14.2 ± 10.5	9.9 ± 2.5

Values are expressed as means ± SD.
* Significantly increased compared with before the intervention ($p < 0.05$).

LB control group. In this study exercise training did not affect fat metabolism in the LB obese women. However, it is not likely that lipolysis and/or fat oxidation would decrease when no exercise training was executed because fat mass did not change and the time span of the study was relatively short. Therefore, lack of a LB obese control group was not expected to affect the interpretation of the outcome of the study.

In our study, the acetate recovery factor was used to correct for label loss in the TCA cycle during the palmitate infusion test (27). The acetate correction is dependent on basal metabolic rate, percentage of body fat, and RER, and needs to be determined in each subject (39). Therefore, measurements of acetate recovery were performed individually both before and after exercise training. At rest, the acetate recovery factor has a large impact on plasma FFA oxidation rates because only ~24% of ^{13}C label was recovered in exhaled breath. During exercise, label recovery was ~62%. If the acetate recovery factor had been ignored, plasma palmitate oxidation would have been underestimated by ~76% at rest and ~38% during exercise. Calculated nonplasma FA oxidation rates at rest before and after the intervention were significantly lower than zero ($p < 0.05$). These negative values suggest that values for plasma palmitate oxidation rates were probably overcorrected by using the acetate correction factor. This may indicate that more ^{13}C acetate is trapped in the TCA cycle than ^{13}C palmitate. One possible pathway for label fixation is the loss of ^{13}C from the TCA cycle in the form of glutamate and glutamine. However, this was not significantly different for palmitate and acetate (28), and therefore is not likely to play a role in the overcorrection of the plasma palmitate oxidation. Further research is needed to explain the overcorrection of the plasma palmitate oxidation by using the acetate recovery factor.

In conclusion, low-intensity exercise training increases relative fat oxidation during exercise but not at rest in UB obese women. In LB obese women, however, low-intensity exercise training of the intensity and frequency used does not affect fat metabolism. Nevertheless, low-intensity exercise training should not necessarily be discouraged in LB obese women because exercise training increases physical fitness and might have positive effects on other health aspects such as blood lipid levels and blood pressure. Moreover, data from the present study do not exclude the possibility that exercise training at another intensity and/or frequency is able to affect fat oxidation in LB obese women.

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